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Published in:
GCB Bioenergy

DOI:
10.1111/gcbb.12549

Publication date:
2019

Citation for published version (APA):
Mangold, A., Lewandowski, I., Möhring, J., Clifton-Brown, J., Krzyzak, J., Mos, M., Pogrzeba, M., & Kiesel, A. (2019). Harvest date and leaf:stem ratio determine methane hectare yield of miscanthus biomass. *GCB Bioenergy*, *11*(1), 21-33. https://doi.org/10.1111/gcbb.12549

Received: 28 April 2018 | Accepted: 4 July 2018

DOI: 10.1111/gcbb.12549

ORIGINAL RESEARCH

WILEY GLOBAL CHANGE BIOLOGY BIOENERGY

Harvest date and leaf:stem ratio determine methane hectare yield of miscanthus biomass

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Funding information
Bundesministerium für Bildung und Forschung, Grant/Award Number: 031B0163

Abstract

The suitability of miscanthus biomass for anaerobic digestion has already been confirmed by several studies. However, it is rarely used as feedstock in biogas plants, mainly due to uncertainty about the optimal harvest regime with regard to the long-term methane hectare yield and resilience of the crop to green cutting. The recommended green-cut date for the only commercially available genotype *Miscanthus* × *giganteus* (*M*×*g*) ranges from September to November. This time-frame is too broad for agricultural practice and needs to be both narrowed down and further specified for different genotypes. The aim of this study was to identify the most suitable harvest window for an autumn green cut of miscanthus, which delivers both a high dry matter and methane yield while securing the long-term productivity of the crop. A further objective was to quantify the effect of genotypic differences, such as leaf to stem ratio, on the substrate-specific biogas and methane yield. For these purposes, a field trial with four genotypes (*M*×*g, GNT1, GNT3, Sin55*) was conducted over 2 years (2016/2017) and harvested at 2-week intervals on three dates between mid-September to mid-October. Methane hectare yield ranged from 3,183 m^3 CH_4 ha^{-1} a^{-1} (*Sin55*) to 5,265 m^3 CH_4 ha^{-1} a^{-1} (*M*×*g*), which is mainly influenced by dry matter yield. The substrate-specific methane yield was higher for the leaf (311.0 ml CH_4 $(g\ oDM)^{-1}$) than the stem fraction (285.1 ml CH_4 $(g\ oDM)^{-1}$) in all genotypes due to lower lignin content of leaves. Of all genotypes, *M*×*g* showed the highest and *Sin55* the lowest nutrient use efficiency. We conclude that miscanthus in Germany should be harvested in October to maximize methane yields and nutrient recycling and minimize yield reduction. Additionally, to increase methane hectare yields even further, future miscanthus breeding should focus on a higher leaf proportion.

KEYWORDS
anaerobic digestion, fibre content, harvest date, methane yield, miscanthus genotypes, nutrient removal

1 | INTRODUCTION

Several studies have shown the suitability of miscanthus biomass for anaerobic digestion (Kiesel & Lewandowski, 2017; Mayer et al., 2014; Wahid et al., 2015). Kiesel and Lewandowski (2017) demonstrated a methane hectare yield potential for *Miscanthus* × *giganteus* of about 6,000 m^3

ha^{-1} year^{-1}. This is within the range of maize, the most common biogas crop in Germany, and therefore an important benchmark for all novel biogas crops (FNR, 2017). A similar amount of agricultural land is required for miscanthus as for maize cultivation to supply a biogas plant (Kiesel, Wagner, & Lewandowski, 2017).

Despite these positive results, miscanthus is rarely used as a substrate for anaerobic digestion in Germany. One reason may be the high establishment costs caused by the labour-intensive and expensive rhizome propagation. However, several studies have shown that a decrease in establishment costs can be expected in the years to come through novel establishment techniques such as seed and collar propagation (Clifton-Brown et al., 2017; Mangold, Lewandowski, Xue, & Kiesel, 2017).

Another reason is the uncertainty about the extent to which the necessary green cut in autumn affects the long-term productivity of the crop. Miscanthus is mainly used for combustion and harvested after winter when lignin and dry matter contents are high (Iqbal & Lewandowski, 2014). This harvest date fits in well with the natural growing cycle of the crop, because it allows the relocation of nutrients to the rhizomes and recycling of nutrients via leaf-fall, thus supporting regrowth in the following year (Cadoux, Riche, Yates, & Machet, 2012).

For use in biogas plants, it is necessary to harvest miscanthus green, i.e. before winter. Later harvests are accompanied by an increase in lignin content in the biomass and thus a decrease in digestibility (Fernandes, Bos, Zeeman, Sanders, & van Lier, 2009). A green cut also leads to higher dry matter yields, as harvest occurs before leaf-fall (Kiesel & Lewandowski, 2017). Schmidt, Lemaigre, Ruf, Delfosse, and Emmerling (2017) found significantly higher dry matter yields from a green cut in autumn (up to 27 t ha^{-1}) than from a brown harvest after winter (22 t ha^{-1}).

However, a green cut allows less time for miscanthus to relocate carbohydrates and nutrients to the rhizome, which can have a potentially negative effect on regrowth in the following year. Fritz and Formowitz (2010) and Kiesel and Lewandowski (2017) showed that an early green cut before or in August negatively affects the long-term productivity of miscanthus. Therefore, for Central European climate conditions, a harvest in September to October is recommended, as both high methane hectare yields and a sufficient green-cut tolerance can be achieved (Kiesel & Lewandowski, 2017). Ruf, Schmidt, Delfosse, and Emmerling (2017) found that a green cut in late September negatively affected the yield the following year, but no physiological effects of a green harvest in November. This range of recommended harvest times (September to November) is too broad for agricultural practice and needs to be further refined.

Biomass quality is not only influenced by harvest time but also by physiological properties, for example proportion of leaf and stem biomass. This has already been established for the combustion of miscanthus biomass, with leaves being less suitable than stems due to their higher content of ash and critical elements (Baxter et al., 2014). For anaerobic digestion, it appears to be the reverse. Wahid et al. (2015) investigated six different harvest dates from August to November and found a significantly higher substrate-specific methane yield (SMY) of the leaf fraction than the stem fraction for the genotypes *M.* × *giganteus* and *Miscanthus sinensis* after 31 days of anaerobic digestion. In addition, they found higher specific methane yields and lower cellulose and lignin contents for *M. sinensis* than for *M.* × *giganteus*. However, Wahid et al. (2015) only investigated 1 year and so were unable to assess the effects on the yield the following year, which is crucial for agricultural practice. Furthermore, the effect of a green harvest on yield in the following year and also on the leaf and stem proportions of the biomass has not been sufficiently investigated.

The aim of this study was to identify the most suitable harvest window for an autumn green cut of miscanthus. This harvest window should enable both a high dry matter and methane yield while securing the long-term productivity of the crop. A further objective was to quantify the effect of genotypic differences, such as leaf proportion, on the substrate-specific and methane hectare yield.

For this purpose, a field trial with four different genotypes was conducted over 2 years and harvested three times at 2-week intervals in the period mid-September to mid-October. In addition to the standard cultivar *M.* × *giganteus*, three novel, seed-based hybrids were tested which are assumed to have improved biomass quality for anaerobic digestion. The dry matter yield (DMY), substrate-specific methane yield (SMY), methane hectare yield (MY) and fibre content were determined for the 2 years 2016 and 2017. The leaf and stem proportions were assessed for each genotype, harvest date and year. Additionally, leaf and stem nutrient contents were measured to quantify the nutrient removal of the green cut.

2 | MATERIALS AND METHODS

2.1 | Field trial

The field trial was conducted on a commercially relevant scale at the University of Hohenheim's research station "Unterer Lindenhof" in south-west Germany (48.4° latitude, 9.2° longitude; approximately 480 m a.s.l.). The location is characterized by a long-term average annual air temperature of 6.8°C and an annual precipitation of 942 mm. The soil, which is classified as a stony marl Rendzina, has a high clay and stone content, and tends to be waterlogged. It can thus be considered to be of low

quality. The climate data for the field trial period (2016–2017) are shown in Supporting Information Table S1.

In the field trial, four genotypes, *M.* × *giganteus* (*M*×*g*), *GNT1*, *GNT3* and *Sin55,* were established in strip plots (width:10.5 m × length: 45.5 m) in a randomized complete block design with four replicates for each genotype, except *GNT1*, which only had three field replications, as the establishment of one field plot was not successful. A detailed description of the four genotypes is given in Table 1.

The planting density was two plants/m^2 with a row distance of 75 cm. The trial was not fertilized during the whole experiment. Weed control was performed annually either mechanically or chemically.

Within the strip plots, a smaller "cutting tolerance trial" was established in a randomized split-plot design, where genotype was the main-plot factor and harvest time was the sub-plot factor. This resulted in 45 plots (three genotypes × four plots × three harvest dates + one genotype × three plots × three harvest dates). Each of those 45 harvest plots had a size of approximately 12 m². The cutting tolerance trial started in the second year after establishment of the miscanthus crop and three different harvest dates were tested. The first harvest date was mid-September (HD 1; 21 September 2016; 18 September 2017), the second 2 weeks later at the beginning of October (HD 2; 4 October 2016/2017) and the third mid-October (HD 3, 17 October 2016/2017).

The harvest procedure was the same for each harvest date in both years. First, the front border of each plot was cut and removed, then eight plants were harvested from the centre of the plot with a "Baural" field trial harvester at a cutting height of 20 cm. The exact area harvested was measured to determine the fresh matter yield (FMY)/ha. To identify the dry matter content (DMC) of the chopped material, a subsample of approximately 1 kg was taken from each plot and dried in a drying cabinet at 60°C to constant weight. The dry matter yield (DMY; t/ha) was estimated based on the FMY and the DMC of the subsample.

Ten randomly selected stems were collected from the remaining borders and separated into leaf and stem fractions. In 2016, 20 stems of the genotype *Sin55* were cut, as ten stems would not have given enough plant material for the analysis. The fractions "leaf" and "stem" were also dried at 60°C to constant weight to identify DMC. After the ten stems had been collected, the remaining borders of each harvest plot were also removed.

2.2 | Biogas batch test

To determine the substrate-specific methane yield (SMY) of the leaf and stem fractions, the dried biomass samples were milled using a cutting mill equipped with a 1-mm sieve (SM 200; Retsch GmbH, Haan, Germany). They were then analysed in a biogas batch test according to VDI guideline 4630. A detailed description of this batch test can be found in Kiesel and Lewandowski (2017). Methane hectare yield was calculated by multiplication of substrate-specific methane yield and the organic dry matter yield.

2.3 | Laboratory analysis

For each laboratory analysis, a subsample was taken from the leaf and stem fractions.

The ash content was determined by incinerating all samples in a muffle kiln at 550°C for 4 hr according to VDLUFA book III, method 8.1 (Naumann & Bassler, 1976/2012).

Lignin, cellulose and hemicellulose content of leaf and stem were analysed by near-infrared spectroscopy (NIRS). Validation and calibration samples were analysed with an ANKOM^{2000} Fiber Analyser and Daisy II Incubator (ANKOM Technology, Macedon, USA). The contents of

TABLE 1 Detailed description of the four genotypes used in the field trial

	Miscanthus × *giganteus* (*M*×*g*)	*GNT1*	*GNT3*	*Sin55*
Type of genotype	Natural hybrid of *Miscanthus sinensis* and *Miscanthus sacchariflorus*	Artificial hybrids of *Miscanthus sinensis* and *Miscanthus sacchariflorus*		*Miscanthus sinensis* genotype
Origin	South-east Asia	Miscanthus breeding programme of Aberystwyth University		
Propagation characteristics	Vegetative propagation via rhizomes or in vitro culture	Seed propagation		
Senescence characteristics	Early senescence	Later senescence than *M*×*g*		Stay-green genotype (delayed senescence compared to *M*×*g, GNT1* and *GNT3*)
Additional information	Currently, single commercially available genotype			High leaf proportion

neutral detergent fibre (NDF), acid detergent fibre (ADF) and acid detergent lignin (ADL) were determined according to VDLUFA book III, method 6.5.1 (NDF), 6.5.2 (ADF), and 6.5.3 (ADL), Naumann & Bassler, 1976/2012;. Table 2 shows the standard error of the NIRS calibration (SEC) and prediction (SEP) and the R^2 of the NIRS calibration and validation. The ADL content is given by the lignin content. Hemicellulose content is determined by subtracting ADF from NDF, cellulose by ADL from ADF.

Nitrogen (N) contents of leaf and stem were analysed using a Vario Max CNS (Elementar Analysensysteme GmbH, Langenselbold, Germany), as described in the VDLUFA Method Book III, method 4.1.2 and DIN ISO 5725.

The phosphorus (P) and potassium (K) contents of leaf and stem were analysed according to the VDLUFA Method Book III, method 10.8.1. For this analysis, 0.5 g of each sample was first dissolved in 8 ml HNO_3, 1 ml H_2O and 5 ml H_2O_2 and then placed in an ETHOS.lab microwave (MLS GmbH, Leutkirch, Germany) for pressure and temperature digestion. Potassium and phosphorus contents were measured with an ICP-OES.

2.4 | Statistical analysis

The experiment was performed in two phases. The first phase consisted of a field trial; in the second phase, samples from the field trial were processed in the laboratory.

The SMY of the leaf and stem fraction were each analysed by a linear mixed model, which considered both field trial and laboratory design (Equation 1).

$$y_{hijkl} = \mu + g_i + d_h + f_j + (gd)_{ih} + (df)_{hj} + (gf)_{ij} + (gdf)_{ihj} + s_l + (gs)_{il} + r_k + e_{hijlk} \quad (1)$$

where y_{hijkl} is the measurement of the *i-th* genotype on the *h-th* harvest date with the *j-th* effect of year in the *l-th* field replication and the *k-th* laboratory replicate. μ is the general effect, g_i is the *i-th* genotype effect *(M×g*; *GNT1*; *GNT3*; *Sin55*), d_h is the main effect of the *h-th* harvest date (HD 1; HD 2; HD 3), f_j is the main effect of the *j-th* year (2016; 2017), $(gd)_{ih}$ is the interaction effect of the *i-th* genotype with the *h-th* harvest date, $(df)_{hj}$ is the interaction of the *h-th* harvest date with the *j-th* year, $(gf)_{ij}$ is the interaction of the *i-th* genotype with the *j-th* year, $(gdf)_{hij}$ is the interaction of the *i-th* genotype with the *h-th* harvest date and the *j-th* year. While all effects described above were taken as fixed, the remaining effects were taken as random. s_l is the random effect of the *l-th* replicate in the first phase (field), r_k is the random effect of the *k-th* replicate in the second phase (laboratory) and e_{hijkl} is the residual error term corresponding to y_{hijkl}. Furthermore, $(gs)_{il}$ is the main-plot error effect associated with main plots of genotype *i* in replicate *l*.

All other traits (DMY, MY, fibre and nutrient content) were measured for both leaf and stem after the first phase; thus for these traits the effects of the *k-th* replicate in the laboratory were dropped from the model in Equation 1.

In all analyses, residuals were graphically checked for normality and homogeneity of variance. For phosphorus (P), log transformation of the data was necessary. Where significant differences were found using an F test, a multiple *t* test (LSD) with $\alpha = 0.05$ was performed. All data analysis was performed using the PROC MIXED procedure of Statistical Analysis Software SAS, version 9.4 (SAS Institute Inc., Cary, NC, USA).

3 | RESULTS

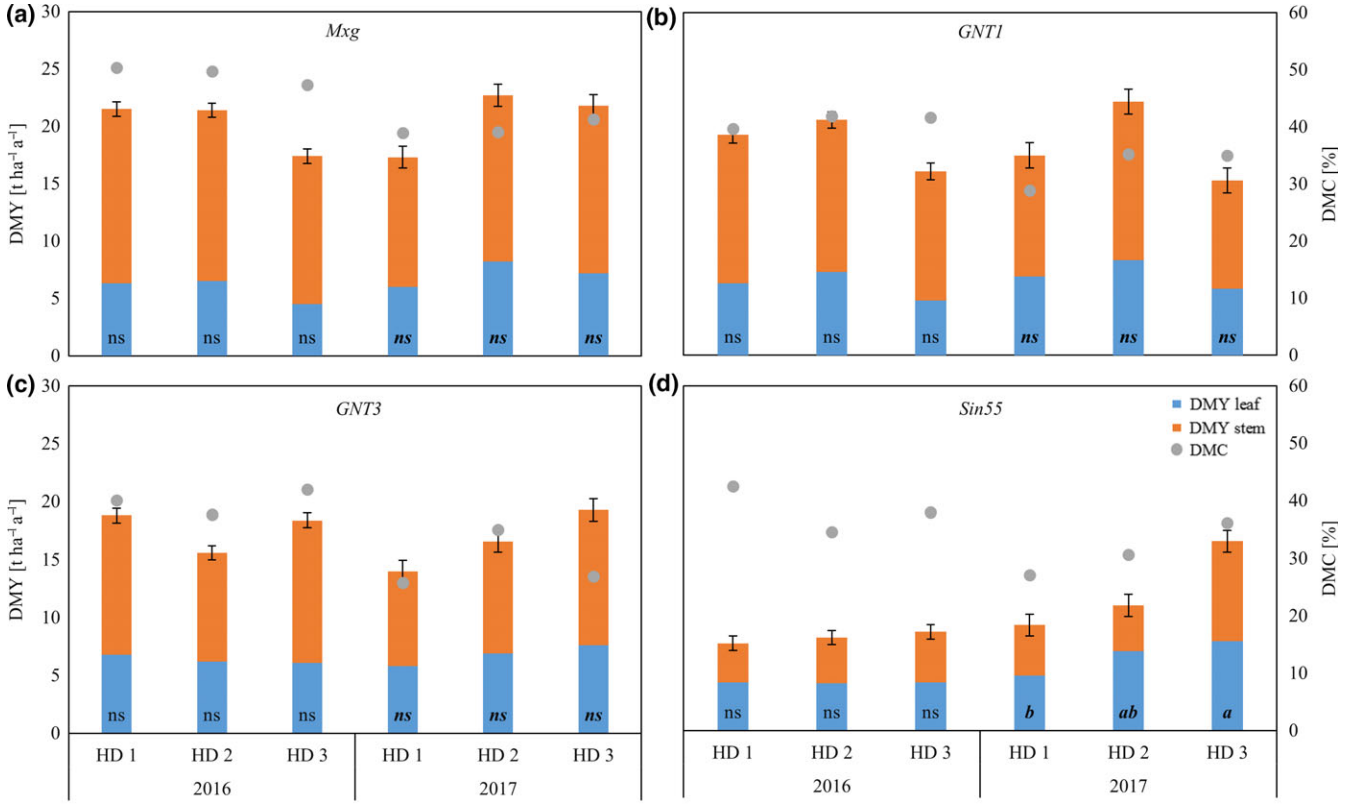
3.1 | Dry matter yield and leaf proportion

Figure 1 shows the DMY and dry matter content (DMC) of the four genotypes on the three harvest dates in 2016 and 2017. The interactions of genotype and year as well as harvest date and year affected dry matter yield of both leaf and stem (Table 3).

Of all four genotypes, *M×g* yielded highest (average: 19.89 t DM ha^{-1} a^{-1}) and *Sin55* lowest (10.83 t DM ha^{-1} a^{-1}) in both years. Both *M×g* and *Sin55* had a higher average yield in 2017 than in 2016; for *GNT1* and *GNT3* it

TABLE 2 NIRS calibration and validation characteristics

	Calibration			Validation		
	Number of samples	Standard error of calibration	R^2	Number of samples	Standard error of prediction	R^2
NDF 2016	181	1.2343	0.9595	25	1.234	0.812
ADF 2016	183	1.3089	0.9615	25	1.271	0.973
ADL 2016	182	0.6764	0.8837	25	0.733	0.887
NDF 2017	195	1.1555	0.9637	45	2.248	0.82
ADF 2017	194	1.1693	0.9695	45	3.77	0.802
ADL 2017	195	0.7153	0.837	45	3.34	0.019



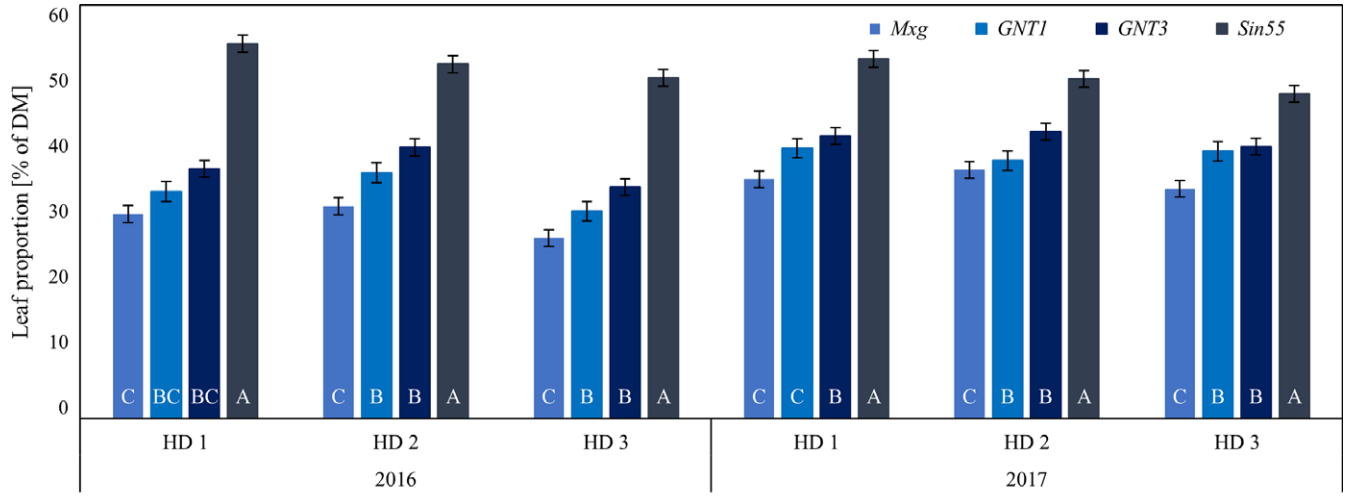
	HD 1		HD 2		HD 3	
	2016	2017	2016	2017	2016	2017
Mxg	A	B	**B**	**A**	*B*	*A*
GNT1	NS	NS	**NS**	**NS**	*NS*	*NS*
GNT3	A	B	**NS**	**NS**	*NS*	*NS*
Sin55	NS	NS	**B**	**A**	*B*	*A*

FIGURE 1 Average dry matter yield (DMY; bars; t ha^{-1} a^{-1}) and dry matter content (DMC; dots; %) of the four genotypes (*M×g* [a], *GNT1* [b], *GNT3* [c], and *Sin55* [d]) over 2 years (2016/2017) on three harvest dates (HD 1: mid-September, HD 2: beginning of October, HD 3: mid-October). The blue section of bars represents leaf proportion, the orange section stem proportion. Different lower-case letters within the bars represent significant differences for DMY between harvest dates for each year and genotype (ns: not significant; standard letters for 2016; bold italic letters for 2017). The lower table presents significant differences between the years (2016/2017) for each harvest date and genotype. Standard letters indicate differences for HD 1, bold letters for HD 2 and italic letters for HD 3. Means with same letters were not significant different from each other. Level of significance was $\alpha = 0.05$. Error bars represent standard error for DMY

TABLE 3 *p*-values for F tests of fixed effects ($\alpha = 0.05$) for dry matter yield (DMY), substrate specific methane yield (SMY), methane hectare yield (MY) (leaf and stem), and the leaf proportion (leaf prop; total crop)

Source	DMY_{leaf}	DMY_{stem}	SMY_{leaf}	SMY_{stem}	MY_{leaf}	MY_{stem}	Leaf prop
Genotype (Geno)	0.0836	<0.0001	<0.0001	<0.0001	<0.0001	0.1273	<0.0001
Harvest date (HD)	0.1435	0.3244	0.0510	0.1449	0.3953	0.0388	0.0002
Year	<0.0001	0.3602	0.8126	0.0048	0.0017	<0.0001	0.0017
HD × year	0.0026	0.0040	0.1625	0.0007	0.0117	0.0759	0.0274
Geno × HD	0.2849	0.2442	0.8218	0.2409	0.1959	0.3869	0.1701
Geno × year	0.00520	<0.0001	0.0829	0.0421	<0.0001	0.0038	0.0058
Geno × HD × year	0.8527	0.6171	0.3387	0.0170	0.2680	0.4184	0.4006

was the reverse. For the genotypes *M×g*, *GNT1* and *GNT3*, the differences in DMY between the three harvest dates within a year were not significant. However, there was a clear trend for all three genotypes that the HD 1 yield was lower, but the HD 2 and 3 yields were similar or slightly higher in 2017 than 2016. Indeed, *Sin55* almost doubled



	HD 1	HD 2	HD 3	HD 1	HD 2	HD 3
Mxg	a	a	b	**ns**	**ns**	**ns**
GNT1	ab	a	b	**ns**	**ns**	**ns**
GNT3	a	ab	b	**ns**	**ns**	**ns**
Sin55	a	ab	b	**a**	**ab**	**b**

FIGURE 2 Average leaf proportion of the four genotypes (*M×g*, *GNT1*, *GNT3* and *Sin55*) over 2 years (2016/2017) on three harvest dates (HD 1: mid-September, HD 2: beginning of October, HD 3: mid-October). The white letters within the bars indicate significant differences in leaf proportion between the genotypes for each harvest date and year. The lower table shows significant differences in leaf proportion between harvest dates for each year (standard letters for 2016, bold letters for 2017) and genotype. Means with same letters were not significant different from each other. Level of significance was $\alpha = 0.05$. Error bars represent standard error for leaf proportion

the yield at HD 2 and 3 in 2017 compared with 2016, while at HD 1 yield level was similar for both years. Additionally, *Sin55*, yielded significantly higher at HD 3 than at HD 1 in 2017 (Figure 1d).

On average, *M×g* had the highest (44.3%) and *GNT3* the lowest (34.7%) DMC. The DMC of all genotypes was higher 2016 than in 2017 (Figure 1). The average DMC of all genotypes was highest at HD 1 (43.1%) in 2016. In 2017, the highest DMCs were recorded at HD 2 (35.0%) and HD 3 (34.9%).

Except for the genotype *Sin55*, the dry matter yield was mainly made up of stems. As shown in Figure 2, the average leaf proportion was lowest in genotype *M×g* (31.7% of DM) and highest in *Sin55* (50.6%). All genotypes (except *Sin55*) had a higher leaf proportion in 2017 than 2016. The leaf proportion was lowest at HD 3 for all genotypes in both years.

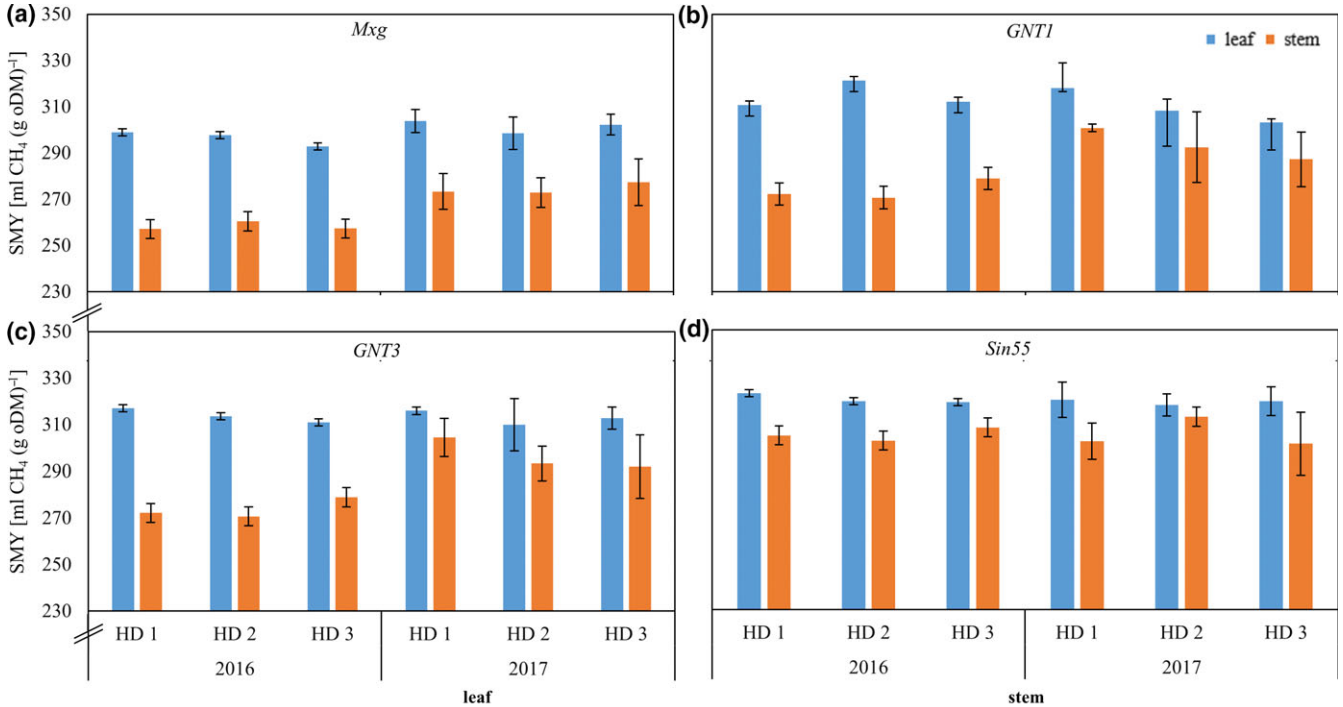
3.2 | Methane yield

The substrate-specific methane yield (SMY) of both leaf and stem biomass was significantly affected by genotype, but not by harvest date (Table 3). The average SMY of all genotypes was 311.0 ml CH_4 (g oDM)$^{-1}$ for the leaf fraction and 285.1 ml CH_4 (g oDM)$^{-1}$ for the stem fraction (Figure 3).

The highest SMY (average of all three HDs) was found in *Sin55* (Figure 3d; leaf: 319.6 ml CH_4 (g oDM)$^{-1}$; stem: 305.1 ml CH_4 (g oDM)$^{-1}$); the lowest was found in *M×g* (leaf: 299.0 ml CH_4 (g oDM)$^{-1}$; stem: 266.3 ml CH_4 (g oDM)$^{-1}$; Figure 3a).

A comparison of the three HDs of each year revealed no clear trends in substrate-specific methane yield. On average, *M×g*, *GNT1* and *GNT3* had higher SMY in 2017 than 2016, while that of *Sin55* was similar in both years.

The methane hectare yield (MY) of leaf and stem fractions was significantly affected by year and genotype × year (Table 3). As shown in Figure 4, average methane hectare yield (MY) was highest for *M×g* (5,265 m³ CH_4 ha^{-1} a^{-1}) and lowest for *Sin55* (3,183 m³ CH_4 ha^{-1} a^{-1}). In all genotypes, the MY was much more strongly influenced by DMY than by SMY. The contribution of the stem fraction to the MY was higher than that of the leaf fraction in all genotypes, except *Sin55*, where the leaf proportion of the DMY was similar to or higher than the stem proportion. The MY of HD 2 and HD 3 was higher in 2017 than 2016 for all genotypes (except *GNT1* at HD 3). The MY of HD 1 was lower in 2017 than 2016 in all genotypes except *Sin55,* where it increased slightly from 2016 to 2017. In 2017, *M×g* and *GNT1* had the highest MY at HD 2, *GNT3* and *Sin55* at HD 3 (significant differences only in *Sin55*).



	leaf						stem					
	HD 1 2016	HD 2 2016	HD 3 2016	HD 1 2017	HD 2 2017	HD 3 2017	HD 1 2016	HD 2 2016	HD 3 2016	HD 1 2017	HD 2 2017	HD 3 2017
Mxg	a	a	b	**ns**	**ns**	**ns**	NS	NS	NS	**NS**	**NS**	**NS**
GNT1	b	a	b	**ns**	**ns**	**ns**	NS	NS	NS	**A**	**AB**	**B**
GNT3	a	ab	b	**ns**	**ns**	**ns**	B	B	A	**A**	**B**	**B**
Sin55	ns	ns	ns	**ns**	**ns**	**ns**	AB	B	A	**A**	**AB**	**B**

FIGURE 3 Average substrate-specific methane yield (ml CH_4/[g oDM]) of leaf (blue bars) and stem (orange bars) biomass of the four genotypes (*M×g* [a], *GNT1* [b], *GNT3* [c], and *Sin55* [d]) over 2 years (2016/2017) on three harvest dates (HD 1: mid-September, HD 2: beginning of October, HD 3: mid-October). The lower table shows significant differences between harvest dates for each year and genotype (2016: standard letters; 2017: bold letters; leaf: lower-case letters; stem: upper-case letters, $\alpha = 0.05$). Means with same letters were not significant different from each other. Error bars represent standard error for SMY

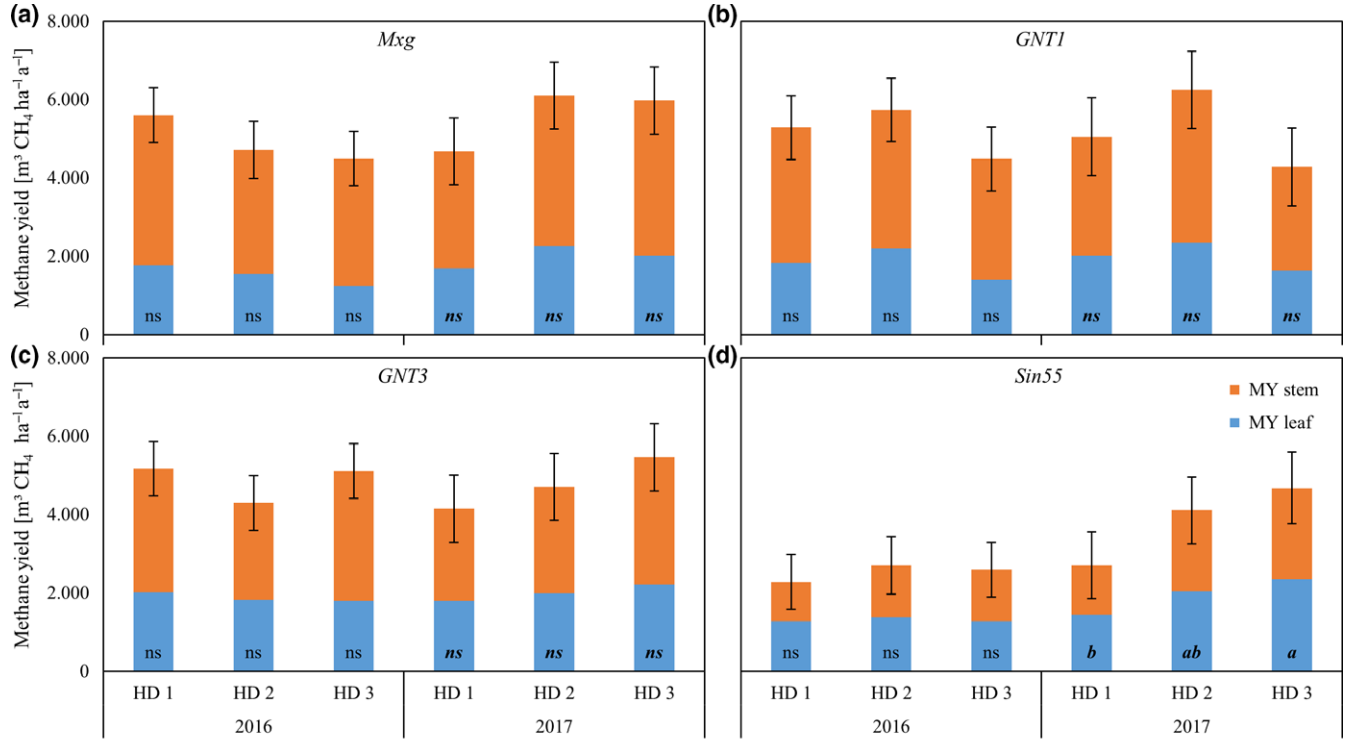
3.3 | Fibre and ash content

The *p*-values for F tests of fixed effects show that all fibre contents, except lignin of leaf fraction, were significantly affected by the interaction of harvest date × year and either genotype or genotype × year (Table 4).

Table 5 shows the fibre (lignin, hemicellulose, cellulose) and ash contents of the four genotypes on the three harvest dates in 2016 and 2017. Genotype *M×g* had the highest average lignin (9.8%) and cellulose (39.2%) content of all genotypes; *GNT1* had the lowest lignin (7.6%) and *Sin55* the lowest cellulose (36.6%) content. However, *Sin55* had the highest (average: 29.7%) hemicellulose content for leaf and stem fraction in both years. *M×g* had the lowest hemicellulose content (average: 25.4%) in both years, except for stem in 2016. Ash content was lowest for *M×g* (5.0%) and highest for *GNT1* (6.1%). For all genotypes and harvest dates in both years, the lignin and cellulose contents were higher in the stem fraction than the leaf fraction (except *GNT1* and *Sin55* at HD 1 in 2017). For hemicellulose and ash contents, it was the reverse (Table 5).

The nutrient removal was significantly affected by the interaction of genotype and year, except for K_{stem} (Table 4). The average removal over all genotypes, harvest dates and years was 115 kg ha^{-1} a^{-1} for nitrogen (N), 257 kg ha^{-1} a^{-1} for potassium (K) and 17 kg ha^{-1} a^{-1} for phosphorus (P). As shown in Figure 5, *GNT1* had the highest nutrient removal of all genotypes (141 kg ha^{-1} a^{-1} N; 301 kg ha^{-1} a^{-1} K; 23 kg ha^{-1} a^{-1} P), *Sin55* the lowest of N (81 kg ha^{-1} a^{-1}) and K (193 kg ha^{-1} a^{-1}), and *M×g* the lowest of P (11 kg ha^{-1} a^{-1}). The average removal of all three nutrients was higher in 2017 than 2016. In all genotypes, N removal was higher by leaves than stems, for K it was higher in stems than leaves, and for P it was balanced between the two fractions.

A comparison of nutrient use efficiency (NUE; biomass produced in kg per nutrient removal in kg) shows that, on average, *M×g* produced the most biomass per removed



	HD 1		HD 2		HD 3	
	2016	2017	2016	2017	2016	2017
Mxg	A	B	**B**	**A**	*B*	*A*
GNT1	NS	NS	**NS**	**NS**	*NS*	*NS*
GNT3	A	B	**NS**	**NS**	*NS*	*NS*
Sin55	NS	NS	**B**	**A**	*B*	*A*

FIGURE 4 Average methane hectare yield (MY; m³ CH_4 ha^{-1} a^{-1}) of the four genotypes (*M×g* [a], *GNT1* [b], *GNT3* [c], *Sin55* [d]) over 2 years (2016/2017) on three harvest dates (HD 1: mid-September, HD 2: beginning of October, HD 3: mid-October). The blue section of bars represents leaf proportion, the orange section stem proportion. Different lower-case letters within the bars represent significant differences for MY between harvest dates for each year and genotype (ns: not significant; standard letters for 2016; bold italic letters for 2017). The lower table presents significant differences between the years (2016/2017) for each harvest date and genotype. Standard letters indicate differences for HD 1, bold letters for HD 2 and italic letters for HD 3. Means with same letters were not significant different from each other. Level of significance was $\alpha = 0.05$. Error bars represent standard error for MY

nitrogen (192.5 kg/kg N), potassium (83.3 kg/kg K) and phosphorus (1878.3 kg/kg P) of all genotypes (results not shown). By contrast, *Sin55*, produced the least biomass per removed nutrient (127.5 kg/kg N; 54.1 kg/kg K); 785.7 kg/kg P). Taken as an average of all genotypes for both years, the NUE was highest at HD 3 for all three nutrients and lowest at HD 1 for K and P and at HD 2 for N (results not shown).

4 | DISCUSSION

The objective of the study was to determine more precisely the optimal harvest date of a green cut of miscanthus, which not only delivers high dry matter and methane yields but also guarantees the long-term productivity of the crop. A further objective was to identify the effects of genotypic differences, such as leaf proportion, on methane hectare yield. The results showed that, for all genotypes, a harvest in October 2016 had no negative effects on the dry matter and methane hectare yields in the following year. The methane hectare yield (MY) was found to be mainly influenced by the stem fraction, due to the higher dry matter yield (DMY) of stems than leaves, except for *Sin55*, which had balanced leaf and stem proportions. Additionally, we determined that the substrate-specific methane yield (SMY) was significantly higher for leaves than for stems in all genotypes.

The following sections discuss these results with a view to identifying the optimal harvest time for miscanthus for utilization in biogas plants. In addition, the genotypic differences of the four genotypes used are analysed to develop recommendations for future miscanthus breeding.

TABLE 4 *p*-values for F tests of fixed effects ($\alpha = 0.05$) of fibre content (cellulose, hemicellulose, lignin), nutrient removal (N, K, P) and ash content

Source	$Cell_{leaf}$	$Cell_{stem}$	$Hemicel_{leaf}$	$Hemicel_{stem}$	$Lignin_{leaf}$	$Lignin_{stem}$	N_{leaf}	N_{stem}	K_{leaf}	K_{stem}	P_{leaf}	P_{stem}	Ash_{leaf}	Ash_{stem}
Genotype (Geno)	0.1740	0.0016	0.0406	<0.0001	0.0030	<0.0001	0.0106	<0.0001	0.4779	<0.0001	0.0032	<0.0001	0.0004	<0.0001
Harvest date (HD)	0.0100	0.0877	0.0049	0.0923	0.0104	0.6099	0.1859	0.6486	0.2928	0.8518	0.0010	0.7344	0.186	0.0088
Year	0.0333	0.0245	<0.0001	0.1813	0.0015	0.4644	<0.0001	0.0226	<0.0001	<0.0001	<0.0001	0.2670	0.0002	<0.0001
HD × year	0.0008	0.0174	0.0276	0.0253	0.1818	0.0002	0.0192	0.0885	0.0005	0.1123	0.2809	0.2868	0.7894	0.0033
Geno × HD	0.5267	0.0838	0.7554	0.3984	0.4758	0.8184	0.1124	0.0344	0.1671	0.0954	0.0019	0.2669	0.3021	0.9921
Geno × year	0.1535	0.8551	<0.0001	0.4388	0.5742	0.8076	<0.0001	<0.0001	<0.0001	0.1087	<0.0001	0.0218	0.2214	0.0008
Geno × HD × year	0.1081	0.0846	0.1182	0.7804	0.4089	0.0391	0.5476	0.1295	0.9073	0.3699	0.0222	0.1629	0.5830	0.8077

4.1 | Optimal harvest date for a green cut of miscanthus

Our study determined that, after 2 years of observation, the optimal harvest date for the genotypes *M×g*, *GNT3* and *Sin55* is mid-October and for *GNT1* the beginning of October. The early cut in mid-September 2016 had a negative yield effect on all genotypes, with a tendency to lower the biomass yield in 2017 at HD 1, except for *Sin55* (Figure 1). The yield was expected to be higher in 2017 than 2016 for all genotypes because 2017 was the third year of the plantation. Although that of *Sin55* was higher in 2017 than 2016, the increase was much lower than expected. It has been observed that yields of miscanthus plantations increase steadily from the establishment year to the 3rd or even 5th year, while the stocks are expanding (Iqbal, Gauder, Claupein, Graeff-Hönninger, & Lewandowski, 2015; Lewandowski, Clifton-Brown, Scurlock, & Huisman, 2000). For this reason, it is not clear how much the yields in this study were determined by plantation age and how much by early harvest regime. However, because the genotypes *M×g* and *GNT3* yielded significantly lower at HD 1 in 2017 than HD 1 in 2016, a harvest of these genotypes in mid-September cannot be recommended. This is in line with the results of Kiesel and Lewandowski (2017) who found the best green-cut tolerance for *M×g* when harvested in mid-October. Schmidt et al. (2017) found a slight decrease in DMY in the second year of green cutting when *M×g* was harvested in September in an older (19 years) stand, but not in a younger (6 years) one. Thus, the long-term effect of an ongoing autumn harvest in a growing miscanthus stand needs to be further investigated, as the 2-year analysis of our study is too short to reach a final conclusion on the best harvest date of miscanthus with its lifetime of up to 20 years. We presume that the later harvest in mid-October provides more time for nutrient relocation and enhances the long lifetime of the stand. Evidence for this can be seen in the nutrient use efficiency (NUE), which was on average highest at HD 3 (mid-October) for all genotypes, after nutrient translocation back to rhizomes has begun.

Although the soil conditions were not optimal (as described above), all four genotypes had satisfactory yields (average: 16.6 t DM ha^{-1} a^{-1}).

In general, the average MY for *M×g* found in our study (5,265 m^3 CH_4 ha^{-1} a^{-1}) is within the range found in other studies, namely 5,000–6,000 CH_4 ha^{-1} a^{-1} (Kiesel & Lewandowski, 2017; Kiesel et al., 2017; Mayer et al., 2014). This is at the lower end of the MY of maize (5,000–7,000 m^3 CH_4 ha^{-1} a^{-1}), as reported by Mayer et al. (2014) and Kiesel et al. (2017). However, it should be noted that MY is mainly influenced by dry matter yield. A comparison of miscanthus and maize grown at the same

TABLE 5 Fibre (lignin, cellulose, hemicellulose) and ash content [% of DM] of the four genotypes on three harvest dates. Significant differences between harvest dates are shown by different lower-case letters (leaf) and upper-case letters (stem), for each genotype for 2016 (standard letters) and 2017 (bold) ($\alpha = 0.05$; ns = not significant). Means with same letters were not significant different from each other

	HD 1 2016		HD 2 2016		HD 3 2016		HD 1 2017		HD 2 2017		HD 3 2017	
Content (%)	**Leaf**	**Stem**	**Leaf**	**Stem**	**Leaf**	**Stem**	**Leaf**	**Stem**	**Leaf**	**Stem**	**Leaf**	**Stem**
M×g												
Lignin	7.8^{a}	12.0^{NS}	7.3^{a}	9.54^{NS}	6.5^{b}	9.5^{NS}	$\mathbf{10.8^{ns}}$	$\mathbf{12.0^{NS}}$	$\mathbf{9.5^{ns}}$	$\mathbf{11.6^{NS}}$	$\mathbf{9.5^{ns}}$	$\mathbf{12.1^{NS}}$
Cellulose	33.2^{b}	44.3^{AB}	34.1^{b}	42.7^{B}	35.7^{a}	46.1^{A}	$\mathbf{29.0^{ns}}$	$\mathbf{47.6^{NS}}$	$\mathbf{31.3^{ns}}$	$\mathbf{47.5^{NS}}$	$\mathbf{31.3^{ns}}$	$\mathbf{47.0^{NS}}$
Hemicell.	31.8^{a}	19.8^{NS}	31.1^{a}	19.7^{NS}	29.4^{b}	19.9^{NS}	$\mathbf{32.1^{a}}$	$\mathbf{20.2^{NS}}$	$\mathbf{30.6^{b}}$	$\mathbf{19.3^{NS}}$	$\mathbf{31.5^{a}}$	$\mathbf{19.4^{NS}}$
Ash	6.9^{ns}	2.6^{NS}	6.8^{ns}	2.4^{NS}	6.5^{ns}	2.5^{NS}	$\mathbf{7.19^{ab}}$	$\mathbf{3.6^{NS}}$	$\mathbf{7.77^{a}}$	$\mathbf{3.1^{NS}}$	$\mathbf{7.16^{b}}$	$\mathbf{3.1^{NS}}$
GNT1												
Lignin	5.9^{ns}	9.6^{NS}	5.2^{ns}	9.2^{NS}	5.3^{ns}	8.8^{NS}	$\mathbf{5.8^{ns}}$	$\mathbf{9.2^{NS}}$	$\mathbf{6.9^{ns}}$	$\mathbf{9.4^{NS}}$	$\mathbf{6.1^{ns}}$	$\mathbf{9.9^{NS}}$
Cellulose	33.9^{ns}	43.9^{A}	33.3^{ns}	42.9^{AB}	32.9^{ns}	40.3^{B}	$\mathbf{32.7^{ns}}$	$\mathbf{44.1^{NS}}$	$\mathbf{32.7^{ns}}$	$\mathbf{45.4^{NS}}$	$\mathbf{33.3^{ns}}$	$\mathbf{44.6^{NS}}$
Hemicell.	32.5^{ns}	20.2^{NS}	33.3^{ns}	19.3^{NS}	32.1^{ns}	19.4^{NS}	$\mathbf{32.7^{ns}}$	$\mathbf{23.1^{NS}}$	$\mathbf{31.6^{ns}}$	$\mathbf{24.6^{NS}}$	$\mathbf{31.9^{ns}}$	$\mathbf{23.8^{NS}}$
Ash	7.2^{ns}	3.3^{NS}	7.6^{ns}	3.4^{NS}	7.2^{ns}	3.4^{NS}	$\mathbf{8.2^{ns}}$	$\mathbf{6.0^{NS}}$	$\mathbf{8.1^{ns}}$	$\mathbf{5.2^{NS}}$	$\mathbf{8.1^{ns}}$	$\mathbf{5.0^{NS}}$
GNT3												
Lignin	6.5^{a}	9.9^{NS}	5.9^{b}	9.8^{NS}	5.7^{b}	9.0^{NS}	$\mathbf{10.2^{ns}}$	$\mathbf{8.7^{B}}$	$\mathbf{6.2^{ns}}$	$\mathbf{9.5^{A}}$	$\mathbf{6.3^{ns}}$	$\mathbf{9.7^{A}}$
Cellulose	34.9^{ns}	43.0^{A}	34.1^{ns}	42.0^{AB}	34.4^{ns}	40.3^{B}	$\mathbf{29.4^{b}}$	$\mathbf{43.0^{NS}}$	$\mathbf{35.2^{ns}}$	$\mathbf{44.6^{NS}}$	$\mathbf{34.4^{ns}}$	$\mathbf{43.1^{NS}}$
Hemicell.	32.1^{ns}	21.3^{B}	32.3^{ns}	23.0^{A}	31.7^{ns}	22.6^{AB}	$\mathbf{32.0^{ns}}$	$\mathbf{22.6^{NS}}$	$\mathbf{31.8^{ns}}$	$\mathbf{23.4^{NS}}$	$\mathbf{31.4^{ns}}$	$\mathbf{22.8^{NS}}$
Ash	6.6^{ns}	3.7^{NS}	6.1^{ns}	3.6^{NS}	6.0^{ns}	3.6^{NS}	$\mathbf{7.4^{ns}}$	$\mathbf{6.3^{A}}$	$\mathbf{7.0^{ns}}$	$\mathbf{5.1^{B}}$	$\mathbf{7.0^{ns}}$	$\mathbf{5.0^{B}}$
Sin55												
Lignin	6.9^{a}	8.2^{NS}	6.0^{b}	8.1^{NS}	5.6^{b}	7.6^{NS}	$\mathbf{11.9^{a}}$	$\mathbf{7.5^{C}}$	$\mathbf{6.1^{b}}$	$\mathbf{8.3^{B}}$	$\mathbf{7.0^{b}}$	$\mathbf{9.4^{A}}$
Cellulose	32.9^{ns}	40.7^{A}	32.5^{ns}	40.3^{A}	32.1^{ns}	37.7^{B}	$\mathbf{28.2^{b}}$	$\mathbf{40.6^{B}}$	$\mathbf{34.8^{a}}$	$\mathbf{43.0^{A}}$	$\mathbf{33.5^{a}}$	$\mathbf{42.6^{AB}}$
Hemicell.	33.7^{ns}	27.1^{NS}	33.0^{ns}	27.8^{NS}	32.6^{ns}	27.7^{NS}	$\mathbf{32.5^{ns}}$	$\mathbf{26.0^{AB}}$	$\mathbf{32.1^{ns}}$	$\mathbf{26.8^{AB}}$	$\mathbf{31.6^{ns}}$	$\mathbf{25.0^{AB}}$
Ash	5.7^{ns}	4.0^{NS}	6.1^{ns}	3.7^{NS}	5.8^{ns}	4.2^{NS}	$\mathbf{6.8^{ns}}$	$\mathbf{5.9^{A}}$	$\mathbf{6.8^{ns}}$	$\mathbf{5.2^{AB}}$	$\mathbf{6.8^{ns}}$	$\mathbf{4.7^{B}}$

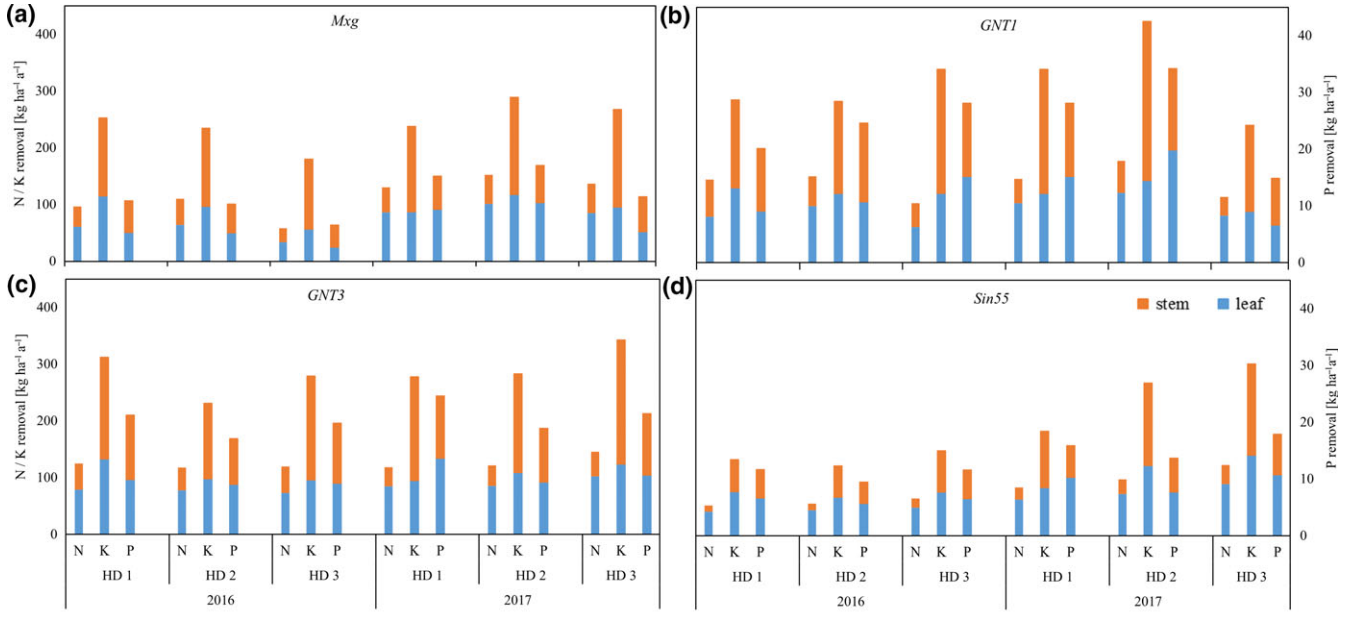
location in 2016/2017 shows that miscanthus would likely have achieved higher MY than maize. This can be deduced from a comparison with maize yields taken from a study by Ehmann, Thumm, and Lewandowski (2018). In this study, the maize plots (which were located adjacent to the field trial in our study) yielded on average approx. 11 t DM ha^{-1} in 2016/2017. A comparison of the DMY for 2016/2017 of this maize with that of the miscanthus in our trial (16.6 t ha^{-1} a^{-1}) reveals a higher DMY for miscanthus.

Additionally, both the production costs and negative environmental impacts are lower for miscanthus than maize biomass under certain conditions, due to lower input requirements for example fertilizer (Wagner et al., this issue). This is mainly a result of the high nutrient use efficiency (NUE) of miscanthus (Cadoux et al., 2012; Lewandowski & Schmidt, 2006). With a view to achieving a high NUE, the latest harvest date in mid-October is preferable. Firstly, the nutrient removal by the biomass is lower. Secondly, the resilience of the crop to a green cut is most likely higher as the nutrients have already partly been relocated from the aboveground biomass to the rhizomes.

As shown by Kiesel and Lewandowski (2017), the nutrient removal of a harvest in mid-October is twice as high as that of a harvest after winter. Thus, to better close nutrient cycles, we recommend the application of digestate in spring to return N, K and P to the field.

When harvested green, miscanthus biomass needs to be ensiled for storage. Therefore, the optimization of the harvest date also has to be considered with regard to the ensiling ability of the biomass. It has been shown that miscanthus biomass is best ensiled when harvested at a dry matter content (DMC) of 35% –40% (Mangold, Lewandowski, Möhring, & Kiesel, this issue). This also indicates that mid-October is the most suitable harvest date.

The lower average DMC found in our study in 2017 can be attributed to the higher accumulated precipitation that year than in 2016. This led to a slower ripening of all genotypes in 2017, resulting in lower average DMCs of 33.3%. However, no recommendations can be provided for optimizing the DMCs through harvest regimes as no clear trends were observed. Generally, it is expected that the DMC increases with senescence and increasing lignin contents of the biomass. Table 5 shows the increase in stem



		HD 1 2016	HD 2 2016	HD 3 2016	HD 1 2017	HD 2 2017	HD 3 2017
Mxg	N	a	a	b	**ns**	**ns**	**ns**
	K	a	ab	b	**ns**	**ns**	**ns**
	P	a	a	b	**ab**	**a**	**b**
GNT1	N	a	a	b	**ns**	**ns**	**ns**
	K	ns	ns	ns	**ab**	**a**	**b**
	P	ns	ns	ns	**a**	**a**	**b**
GNT3	N	ns	ns	ns	**ns**	**ns**	**ns**
	K	a	b	ab	**ns**	**ns**	**ns**
	P	ns	ns	ns	**ns**	**ns**	**ns**
Sin55	N	ns	ns	ns	**ns**	**ns**	**ns**
	K	ns	ns	ns	**ns**	**ns**	**ns**
	P	ns	ns	ns	**ns**	**ns**	**ns**

FIGURE 5 Average nutrient removal (kg ha^{-1} a^{-1}) of the four genotypes (*M×g* [a], *GNT1* [b], *GNT3* [c], *Sin55* [d]) over 2 years (2016/2017) on three harvest dates (HD 1: mid-September; HD 2: beginning of October; HD 3: mid-October). The blue part of the bars indicates the proportion of nutrient removal by the leaves, the orange part the proportion of nutrient removal by stems. The lower table shows significant differences between harvest dates for 2016 (standard letters) and 2017 (bold letters) for each genotype for nitrogen (N), potassium (K) and phosphorus (P). Means with same letters were not significant different from each other. Level of significance was $\alpha = 0.05$

and leaf lignin contents with later harvest for all genotypes. However, the DMC of *GNT3* at HD 3 2017 (shown in Figure 1c) neither followed that trend nor could it be explained by weather conditions. Therefore, further analysis is necessary to clarify the influence of weather conditions, for example on DMC, over a longer time period.

4.2 | Recommendations for miscanthus breeding

Our results demonstrated significant differences in leaf proportion between the four genotypes at all harvest dates in both years (Figure 2). *Sin55* had the significantly highest (50% of DM) leaf proportion, followed by *GNT3* and *GNT1*. *M×g* had the significantly lowest (30% of DM) leaf proportion (Figure 2). A glance at the average SMY of the genotypes reveals a positive correlation between leaf proportion and SMY for all genotypes. This is most likely due to lower lignin contents of leaves than stems (Table 5) as, according to Fernandes et al. (2009), lignin has a low biodegradability. Wahid et al. (2015) also found significantly lower lignin contents of leaves than stems, resulting in a significantly faster biomethane production during the first 31 days of fermentation. Our study found similar results (within the first 10 days) for digestion velocity in 2016 for all genotypes and in 2017 for *M×g* (results not shown). However, as this result could not be confirmed for the other genotypes in 2017, the effect of leaf and stem proportion on digestion velocity needs to be investigated over more years. By contrast, our study confirmed the results of other studies (Kiesel & Lewandowski, 2017; Wahid et al., 2015) that methane hectare yield is mainly

influenced by dry matter yield rather than by SMY. For this reason, miscanthus with a higher leaf proportion in its biomass—and thus higher leaf proportion in its DMY—is more favourable for utilization in anaerobic digestion. In combination with higher SMY, this would result in higher MY and most likely also improve the digestibility. Various studies have revealed a negative correlation between lignin content of biomass and its SMY (von Cossel, Möhring, Kiesel, & Lewandowski, 2017). However, from the results of our study, we cannot conclude that lignin content alone is a suitable criterion for breeding biogas miscanthus. Although leaves had lower lignin contents than stems, leading to higher SMYs, this was not true of the average lignin content of the genotypes. For example, *GNT1* had the lowest lignin content of all genotypes (Table 5) but did not yield the highest average SMY. Additionally, for all genotypes except *GNT1*, the harvest date with the lowest lignin content did not result in the highest SMY. Thus, we cannot recommend selecting miscanthus genotypes for anaerobic digestion on the basis of lignin content alone. Instead, a selection on the basis of hemicellulose content would appear more reasonable as there was a positive correlation between the average hemicellulose content and average SMY in each genotype.

The stay-green genotype *Sin55* was expected to be most suitable for anaerobic digestion on account of late senescence, which leads to lower dry matter contents. It was thus expected to have lower lignin contents, in turn leading to a better digestibility. These expectations, however, were not confirmed by our study. Despite *Sin55* having the highest SMY, it did not have the highest methane hectare yield. This was mainly due to its low DMY, especially in 2016. This leads us to the hypothesis that *Sin55* has a delayed establishment compared with the other three genotypes, resulting in lower yields. We expect that *Sin55* was still in the process of establishing and assume that in 2018 its MY will be similar to that of the other genotypes. In their study, Wahid et al. (2015) demonstrated a higher DMY and biomethane potential, but lower lignin content, for a *M. sinensis* genotype than for *M*×*g*. In addition, they showed that the *M. sinensis* genotype had a slightly higher digestion velocity than *M*×*g* during 35-day fermentation. Our analysis also showed a tendency for higher digestion velocity in *Sin55* than *M*×*g* (results not shown). Better digestibility of the biomass is preferable, as it saves costs for additional pretreatment. Several studies have recommended the pretreatment of miscanthus biomass to gain higher methane yields (Frydendal-Nielsen et al., 2016; Zheng, Zhao, Xu, & Li, 2014), but pretreatment is usually energy- and cost-intensive (Zheng et al., 2014). Therefore, it is likely that, for the genotype *M*×*g*, higher methane yields and thus higher revenues will not necessarily lead to higher profits, as higher costs for pretreatment are incurred. By contrast, *Sin55* could be more profitable, as it requires less pretreatment. Thus, we conclude that stay-green *M. sinensis* genotypes, such as *Sin55*, are most suitable for anaerobic digestion, due to their high SMY and low lignin contents.

In terms of nutrient use efficiency (NUE), *M*×*g* is the most suitable and *Sin55* the least suitable genotype for the supply of low-input biomass for anaerobic digestion, as *M*×*g* produced the most and *Sin55* the least biomass per removed unit of nitrogen, potassium and phosphorus. This is most likely mainly due to the higher yields of *M*×*g* compared with the other genotypes.

In conclusion, this study determined that for the miscanthus genotypes *M*×*g*, *GNT1*, *GNT3* and *Sin55*, the most suitable harvest date for high methane yields is October. A late cut in October is also the most favourable in terms of nutrient use efficiency. In addition, the study found that the leaf fraction of miscanthus biomass produced significantly higher substrate-specific methane yields than the stem fraction, which we attribute to the lower lignin content of leaves. For this reason, future miscanthus breeding should focus on genotypes with a higher leaf proportion (e.g. *M. sinensis* genotype *Sin55*) to increase methane hectare yields.

ACKNOWLEDGEMENTS

This study was supported by the German Federal Ministry of Education and Research within the framework of the FACCE SURPLUS CALL (funding code: 031B0163). The hybrids *GNT1*, *GNT3* and *Sin55* were selected by Terravesta Ltd., from the UK-led miscanthus breeding programme, mainly supported by the UK's Biotechnology and Biological Sciences Research Council (BBSRC) and Department for Environment, Food and Rural Affairs (DEFRA) through the GIANT-LINK project (LK0863).The authors are grateful to the research station "Unterer Lindenhof", particularly Peter Weckherlin, for the management of the field trial. Additionally, we are grateful to the laboratory staff, especially Dagmar Mezger and Martin Zahner, for analysis of the biomass. Particular thanks go to Nicole Gaudet for proofreading the manuscript.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

How to cite this article: Mangold A, Lewandowski I, Möhring J, et al. Harvest date and leaf:stem ratio determine methane hectare yield of miscanthus biomass. *GCB Bioenergy*. 2018;00:1–13. https://doi.org/10.1111/gcbb.12549